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ORIGINAL ARTICLE

Association between genetic polymorphisms of inflammatory response genes and the risk of ovarian cancer

Zhi-Hui Lu*, Xi-Juan Gu, Ke-Zhi Shi, Xuan Li, Dian-Dian Chen, Li Chen

Department of Oncology, The First Affiliated Hospital of Nanchang University, Nanchang City, Jiangxi Province, 330006, China

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Background/Purpose: Inflammation plays an important role in promoting ovarian tumorigenesis and cancer progression. However, the relationship between polymorphisms in inflammatory response genes and risk of ovarian cancer remains poorly understood. In this study, we investigated the association of *PPARG* Pro12Ala, *IL6*-174G/C, E-selectin S128R, *NFKB1*-94 ins/del, *NFKBIA*-826C/T, and *ICAM-1* K469E polymorphisms with ovarian cancer risk in a Chinese population.
Methods: Genotyping of the polymorphisms was performed on 687 cases and 687 controls employing the PCR-RFLP technique, and the logistic regression model was used to measure the risk association.
Results: A significantly increased risk association was observed for the heterozygous genotypes of *PPARG* [odds ratio (OR) = 1.52, 95% confidence interval (CI) = 1.01–2.29] and E-selectin (OR = 1.77, 95% CI = 1.07–2.93) polymorphisms, as well as the homozygous ins/ins genotype of *NFKB1* polymorphism (OR = 1.39, 95% CI = 1.00–1.92). By contrast, *ICAM-1* KE genotype was associated with a decreased ovarian cancer risk (OR = 0.77, 95% CI = 0.60–0.98). In addition, the *NFKB1* del/del + *NFKBIA* TT combination was also found to be associated with a decreased ovarian cancer risk, with OR = 0.12 (95% CI = 0.01–0.95). The associations of the *NFKB1* and *ICAM-1* polymorphisms replicated the findings of previous reports, assuring the reliability of the results obtained.
Conclusion: *NFKB1* and *ICAM-1* polymorphisms could serve as useful ovarian cancer risk prediction biomarkers for the Chinese population, while the utility of *PPARG* and E-selectin polymorphisms as biomarkers requires further confirmation in independent ovarian cancer cohorts.

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

* Corresponding author. Department of Oncology, The First Affiliated Hospital of Nanchang University, Nanchang City, Jiangxi Province, 330006, China.
E-mail address: zhihui0992@gmail.com (Z.-H. Lu).

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Introduction

Ovarian cancer is one of the leading forms of cancer, both in China and worldwide.[1] Each year, there is an estimated 225,500 new incidences of ovarian cancer cases globally.[2] The lack of effective screening methods causes >70% of ovarian cancer patients to be diagnosed at late stages, which leads to the low 5-year survival rate and high mortality rate of the disease. Worldwide, ovarian cancer contributes to 114,000 deaths annually.[3] A method for the identification of individuals at a higher risk of the cancer is therefore necessary for improving ovarian cancer screening strategy.

The principal risk factors for the occurrence of ovarian cancer are germ line mutations in the *BRCA1* or *BRCA2* genes.[4] However, the carriers of such mutations account for only a small portion of total ovarian cancer cases, and a substantial proportion of ovarian cancer risk among sporadic cases remained unexplained. It has been suggested that common low penetrance genetic variations could confer moderate risk to ovarian cancer cases without a heritable basis.[5] Single nucleotide polymorphisms of genes involved in cancer-related pathways represent the candidates of such genetic variations.

Inflammation has been established as an underlying cause in the development of many cancers.[6] Several events which delay inflammation in the ovaries, such as childbirth and oral contraceptive use, have been associated with a reduced risk of ovarian cancer.[7] On the contrary, events which cause inflammation, such as endometriosis, have been suggested to increase the risk of ovarian cancer.[8] It has been thought that inflammation could induce and activate several oxidant-generating enzymes, which can lead to damages in the DNA. Given the important link between inflammation and ovarian carcinogenesis, inherited variation in inflammatory response genes could affect ovarian cancer susceptibility.

Polymorphisms in several inflammatory response genes, such as *PPARG* Pro12Ala polymorphism, *IL6* -174G/C polymorphism, E-selectin S128R (A561C) polymorphism, *NFKB1*-94 ins/del polymorphism, *NFKBIA* -826C/T polymorphism, and *ICAM-1* K469E polymorphism, have been found to be associated with various cancers.[9–16] However, the involvement of these polymorphisms in ovarian cancer in the Chinese population has not been well established. To address this matter, we investigated the association between the six inflammatory response genetic polymorphisms mentioned above and the risk of ovarian cancer in a Chinese population.

Materials and methods

Study participants

The study was approved by the Medical Research Ethics Board of the First Affiliated Hospital of Nanchang University, Nanchang City, China and all samples were collected from the above hospital from July 2010 to December 2013. A total of 687 females who were histopathologically confirmed ovarian cancer patients and 687 controls were recruited into the study. Controls were disease-free individuals without cancers and age-matched to the cases in terms of frequency. Blood samples were collected from the participants after obtaining written informed consent.

Genotyping

Genomic DNA was extracted from the blood samples obtained using EasyPure Blood Genomic DNA Kit (TransGen Biotech, Beijing, China) according to the manufacturer's protocol. The genomic DNA obtained was then used in polymerase chain reaction (PCR). The genetic polymorphisms were detected by the PCR-restriction fragment length polymorphism (RFLP) method. Researchers were blinded to the identity and the case–control status of the samples. For all polymorphisms, ~10% of the samples were chosen at random and sequenced to confirm the genotypes.

PPARG Pro12Ala polymorphism

The PCR primers of *PPARG* Pro12Ala polymorphism used were 5′-GCC AAT TCA AGC CCA GTC-3′ and 5′-GAT ATG TTT GCA GAC AGT GTA TCA GTG AAG GAA TCG CTT TCC-3′. The PCR condition was 5 minutes of initial denaturation at 94°C, followed by 35 cycles of 1 minute at 94°C, 30 seconds at 60°C, 30 seconds at 72°C, and a final extension for 5 minutes at 72°C. The PCR amplification produced fragment of 270 bp in size. BstUI restriction enzyme was then used to digest the 270 bp fragment for determination of genotype. The CC genotype remained uncut after restriction digestion. The GG genotype gave a 227 bp and a 43 bp fragment, and heterozygous genotype (GC) genotype was detected by the presence of all the above bands in agarose gel.

IL6-174G/C polymorphism

The PCR condition used for *IL6* -174G/C polymorphism was 5 minutes of initial denaturation at 94°C, followed by 35 cycles of 45 seconds at 94°C, 30 seconds at 58°C, 30 seconds at 72°C, and a final extension for 5 minutes at 72°C. The primers used were 5′-TGA CTT CAG CTT TAC TCT TGT-3′ and 5′-CTG ATT GGA AAC CTT ATT AAG-3′. The PCR reaction gave an amplification product of 161 bp in size. The fragment was then digested by Hsp 92II restriction enzyme. The GG genotype remained 161 bp on agarose gel as it was uncut by the restriction enzyme. However, the restriction digestion produced two smaller fragments, which were 118 bp and 43 bp in size, for CC genotype. In addition, heterozygous genotype (GC) was found to have all the fragments detected in homozygous genotypes, which were fragments with sizes of 161 bp, 118 bp, and 43 bp.

E-selectin S128R (A561C) polymorphism

The E-selectin S128R (A561C) polymorphism was genotyped by first amplifying the region of interest under the following conditions: 10 minutes of initial denaturation at 94°C, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 60.5°C, 30 seconds at 72°C, and a final extension for 10 minutes at 72°C. The PCR primers used were 5′-ATGGCACTCTGTAGGACTGCT-3′ and 5′-GTCTCAGCTCACGATCACCAT-3′. The

357 bp fragment generated was digested by using PstI restriction enzyme. The AA genotype was cleaved into 219 + 138 bp fragments, whereas the CC genotype was not cleaved (357 bp). Heterozygotes (AC) showed all the above bands on agarose gel.

NFKB1-94 ins/del polymorphism

The PCR condition used for *NFKB1*-94 ins/del polymorphism was 5 minutes of initial denaturation at 94°C, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 58°C, 50 seconds at 72°C, and a final extension for 7 minutes at 72°C. The primers used were 5′-TGG GCA CAA GTC GTT TAT GA-3′ and 5′-CTG GAG CCG GTA GGG AAG-′3. PflMI restriction enzyme was used to restrict the PCR product. For ins/ins genotype, a 281 bp fragment was observed on agarose gel upon digestion, as no restriction site was found in the fragment. A 240 bp and a 45 bp fragment were seen for the del/del genotype, while a 281 bp, a 240 bp, and a 45 bp fragment were found in the Ins/del genotype after restriction enzyme digestion.

NFKBIA-826C/T polymorphism

The *NFKBIA* -826C/T polymorphism was genotyped by first amplifying the region of interest by using the following PCR primers: 5′-GGT CCT TAA GGT CCA ATC G-3′ and 5′-GTT GTG GAT ACC TTG CAC TA-3′. The PCR was performed with 10 minutes of initial denaturation at 94°C, followed by 35 cycles of 1 minute at 94°C, 30 seconds at 59.5°C, 45 seconds at 72°C, and a final extension for 5 minutes at 72°C.This produced an amplicon of 200 bp in size. Restriction enzyme digestion was performed with BfaI. The TT genotype was uncut by the enzyme and remained 200 bp, but the TT genotype was cleaved into 180 + 20 bp bands. Heterozygotes showed all three bands on agarose gel.

ICAM-1 K469Epolymorphism

The region containing the *ICAM-1* K469E polymorphism was amplified by using 5′-GGA ACC CAT TGC CCG AGC-3′ forward primer and 5′-GGT GAG GAT TGC ATT AGG TC-3′ reverse primer, which produced a product of 223 bp. The PCR condition comprised of 5 minutes of initial denaturation at 94°C, followed by 35 cycles of 45 seconds at 94°C, 30 seconds at 59°C, 45 seconds at 72°C, and a final extension for 5 minutes at 72°C. BstUI restriction enzyme was then used to cleave the fragment. The AA genotype did not contain the restriction site of the enzyme, and remained 223 bp on agarose gel after the reaction. However, the GG genotype was cleaved into 136 + 87 bp bands, and heterozygotes (AG) contained all the bands (223 + 136 + 87 bp).

Statistical analysis

SPSS Statistics (version 19; SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The difference between cases and controls in terms of demographic characteristics and genotype distribution were calculated by using χ^2 or Student *t* test, whenever appropriate. Binary logistic regression models were used to calculate the odds ratios (ORs) and their 95% confidence intervals (CIs) for measuring of the association of the polymorphisms and ovarian cancer risk, with the wild type genotype served as the reference. All statistical analyses were performed with $p < 0.05$.

Results

Characteristics of cases and controls

The demographic characteristics of the cases and controls are shown in Table 1. The mean and median ages of the cases were 60.0 ± 8.78 years and 64 years respectively, whereas those of the controls were 59.5 ± 8.75 years and 60 years. No significant difference was observed between cases and controls in terms of age ($p = 0.33$). Four hundred and thirty three of the cases were never smokers and 254 were ever smokers. However, 451 controls and 236 controls were never smokers and ever smokers, respectively. The difference in the number of never and ever smokers between cases and controls was also not statistically significant ($p = 0.31$). In terms of oral contraceptive use, 505 cases were never users and 182 were ever users, in contrast to 493 never users and 194 ever users in controls. Similarly, the difference in oral contraceptive use between cases and controls was not statistically significant ($p = 0.47$).

Genotypic distribution of the polymorphisms

The genotypic distribution of the polymorphisms is shown in Table 2. For the *PPARG* polymorphism, 624 cases, 62 cases, and one cases had Pro/Pro, Pro/Ala, and Ala/Ala genotypes respectively, in comparison to 643 controls, 42 controls, and two controls. No significant difference was observed between cases and controls in the distribution of Pro/Pro ($p = 0.06$) and Ala/Ala ($p = 0.57$) genotypes. However, the Pro/Ala genotype appeared to be significantly different between cases and controls ($p = 0.04$), with a higher frequency in the cases.

For the *IL6* polymorphism, 684 cases and 676 controls had GG genotype. However, four cases and 11 controls had GC genotype. The difference between cases and controls in these genotypes were not statistically significant ($p = 0.08$). None of the participants studied had the CC genotype.

Table 1 Characteristics of cases and controls.

Characteristic	Case ($n = 687$)	Control ($n = 687$)	*p*
Age (y)			
Mean ± SD	60.0 ± 8.78	59.5 ± 8.75	0.33
Median	64	60	
Smoking habit			
Never smoker	433	451	0.31
Ever smoker	254	236	
Oral contraceptive			
Never user	505	493	0.47
Ever user	182	194	

Table 2 Genotypic distribution of the six polymorphisms.

Genotype	Case (n = 687)	Controls, (n = 687)	p
PPARG			
Pro/Pro	624	643	0.06
Pro/Ala	**62**	**42**	**0.04**
Ala/Ala	1	2	0.57
IL6			
GG	683	676	0.08
GC	4	11	0.08
CC	0	0	1.00
E-selectin			
AA	**662**	**644**	**0.03**
AC	**25**	**43**	**0.03**
CC	0	0	1.00
NFKB1			
Del/Del	221	253	0.07
Del/Ins	351	339	0.51
Ins/Ins	115	95	0.13
NFKBIA			
CC	486	478	0.64
CT	181	190	0.58
TT	20	19	0.87
ICAM-1			
KK	209	180	0.08
KE	**322**	**362**	**0.03**
EE	156	145	0.47

Significant p values are shown in bold.

Similar to the *IL6* polymorphism, none of the participants studied had the variant genotype (CC) for E-selectin S128R (A561C) polymorphism. Six hundred and sixty two cases had AA genotype, and only 25 had AC genotype. However, 644 controls and 43 controls had AA and AC genotypes, respectively. The difference between cases and controls in the genotypic distribution of AA and AC genotypes was statistically significant, with $p = 0.03$. The AA genotype was overrepresented in the cases, and the AC genotype was overrepresented in the control group.

Meanwhile, for *NFKB1* polymorphism, 221 cases and 253 controls had del/del genotype. However, 351 cases and 339 controls had the heterozygous del/ins genotype, and 115 cases and 95 controls had the ins/ins genotype. Statistically significant difference between cases and controls was not observed for the polymorphism. The p values of the del/del, del/ins and ins/ins genotypes were $p = 0.07$, $p = 0.51$, and $p = 0.13$, respectively.

Four hundred and eighty six cases had the CC genotype for the *NFKBIA* polymorphism, while 181 cases and 20 cases had CT and TT genotypes, respectively. By comparison, 478 controls, 190 controls, and 19 controls had the three respective genotypes. The differences between cases and controls in the distribution of the CC, CT, and TT genotypes were not statistically significant, with $p = 0.64$, $p = 0.58$, and $p = 0.87$, respectively.

However, the frequencies of KK, KE, and EE genotypes for the *ICAM-1* polymorphism were in 209 cases, 322 cases, and 156 cases, and in 180 controls, 362 controls, and 145 controls. Significant difference was not observed between cases and controls in the frequency distribution of the KK ($p = 0.08$) and EE ($p = 0.47$) genotypes. However, a significant difference was observed for the heterozygote KE genotype ($p = 0.03$), which is overrepresented in the control group.

All the genotypic distribution followed Hardy–Weinberg equilibrium ($p > 0.05$).

Risk association of the polymorphisms

The association of the inflammatory gene polymorphisms with ovarian cancer risk was measured in OR units, with the wild type genotype served as the reference. The risk association was summarized in Table 3. For the *PPARG* polymorphism, the Pro/Ala genotype was found to be significantly associated with an increased ovarian cancer risk ($p = 0.04$), with OR = 1.52, 95% CI = 1.01–2.29. The Ala/Ala genotype was not associated with the risk of ovarian cancer significantly ($p = 0.59$). Similarly, no significant risk association was observed for the GC genotype of the *IL6* polymorphism ($p = 0.08$). The risk association of the CC genotype could not be measured because none of the study participants carried the genotype. For the E-selectin polymorphism, a significantly increased ovarian cancer risk association was observed for the AC genotype ($p = 0.03$), with OR = 1.77 (95% CI = 1.07–2.93), and similar to the *IL6* polymorphism, the association of the variant genotype could not be calculated. The *NFKB1* polymorphism, however, was found to be associated with ovarian cancer risk, with the ins/ins genotype increasing the risk by 39% (OR = 1.39, 95% CI = 1.00–1.92), at

Table 3 Risk association of the polymorphisms with ovarian cancer.

Genotype	Case (n = 687)	Controls (n = 687)	OR (95% CI)	p
PPARG				
Pro/Pro	624	643	Reference	—
Pro/Ala	**62**	**42**	**1.52 (1.01–2.29)**	**0.04**
Ala/Ala	1	2	0.52 (0.05–5.70)	0.59
IL6				
GG	683	676	Reference	—
GC	4	11	0.36 (0.11–1.14)	0.08
CC	0	0	—	—
E-selectin				
AA	662	644	Reference	—
AC	**25**	**43**	**1.77 (1.07–2.93)**	**0.03**
CC	0	0	—	—
NFKB1				
Del/Del	221	253	Reference	—
Del/Ins	351	339	1.19 (0.94–1.50)	0.15
Ins/Ins	**115**	**95**	**1.39 (1.00–1.92)**	**0.05**
NFKBIA				
CC	486	478	Reference	—
CT	181	190	0.94 (0.74–1.19)	0.59
TT	20	19	1.04 (0.55–1.96)	0.92
ICAM-1				
KK	209	180	Reference	—
KE	**322**	**362**	**0.77 (0.60–0.98)**	**0.04**
EE	156	145	0.93 (0.69–1.25)	0.62

Significant p values are shown in bold.

borderline significance ($p = 0.05$). Nonetheless, no significant association was observed for the heterozygous del/ins genotype ($p = 0.15$). For the *NFKBIA* polymorphism, none of the genotypes was observed to be associated with the risk of ovarian cancer ($p = 0.59$ for CT genotype and $p = 0.92$ for TT genotype). Finally, for the *ICAM-1* polymorphism, a significant decreased risk association was observed for the KE genotype, with OR = 0.77 (95% CI = 0.60–0.98; $p = 0.04$). Despite this, significant association was not observed for the variant EE genotype ($p = 0.62$).

Combinations of NFKB1 and NFKBIA genotypes and their association with ovarian cancer risk

Because *NFKB1* and *NFKBIA* share the same signaling pathway, we investigated the association of *NFKB1* and *NFKBIA* combination genotypes with the risk of ovarian cancer. The association is shown in Table 4. The numbers of cases who had the *NFKB1* del/del + *NFKBIA* CC, *NFKB1* del/ins + *NFKBIA* CC, *NFKB1* ins/ins + *NFKBIA* CC, *NFKB1* del/del + *NFKBIA* CT, *NFKB1* del/ins + *NFKBIA* CT, *NFKB1* ins/ins + *NFKBIA* CT, *NFKB1* del/del + *NFKBIA* TT, *NFKB1* del/ins + *NFKBIA* TT, and *NFKB1* ins/ins + *NFKBIA* TT genotypes were 161, 246, 79, 59, 93, 29, 1, 12, and 7 respectively, while those of the controls were 172, 241, 65, 72, 93, 25, 9, 5, and 5. Of these, significant difference between cases and controls was observed only for the *NFKB1* del/del + *NFKBIA* TT genotype. The *NFKB1* del/del + *NFKBIA* TT was overrepresented in controls, and the combination genotype was therefore associated with a reduced ovarian cancer risk, with OR = 0.12 (95% CI = 0.01 0.95), $p = 0.04$.

Discussion

The link between inflammation and cancer development has been well established.[6] However, studies investigating the relationship between inflammatory gene polymorphisms and cancer risk have generated contradictory findings.[9–16] The allelic distributions of various polymorphisms could vary geographically and ethnically, thus leading to the discordant findings between these polymorphisms and cancer risk. In this study, we reported the association between six inflammatory gene polymorphisms and ovarian cancer risk in a Chinese population. Our analysis showed that three of the polymorphisms were associated with an increased ovarian cancer risk, and one polymorphism was associated with a decreased ovarian cancer risk.

PPARG encodes for PPAR-γ, a member of the nuclear hormone receptor super family which has been shown to play a pivotal role in regulating cell differentiation, cell growth, glucose, and lipid metabolism, and several other processes known to be important in carcinogenesis.[17,18] Activation of PPAR-γ causes a series of reactions that regulates the production of proinflammatory cytokines, including interleukin (IL)-6.[19] The variant allele of the *PPARG* Pro12Ala polymorphism has been shown to be associated with a reduced transcriptional activity of PPAR-γ.[20] As such, this polymorphism has been hypothesized to contribute to an increased cancer risk. Our findings indicate that the Pro/Ala genotype of the *PPARG* Pro12Ala polymorphism was associated with an increased ovarian cancer risk. However, the association was absent for the variant Ala/Ala genotype. Such an observation could be due to the complex regulatory role of PPAR-γ on cancer-related cytokines production (such as IL-6) during carcinogenesis, although this assumption requires further investigation. Considering the complex relationship between PPAR-γ and IL-6, we also investigated the association between *IL6* polymorphism with ovarian cancer risk. The *IL6*-174G/C promoter polymorphism was selected as it harbors binding sites for multiple transcription factors and the polymorphism has been known to regulate IL-6 protein expression *in vivo* and *in vitro*.[21] We found that the *IL6* polymorphism was associated with a reduced risk of cancer, but without statistical significance although the *p* value was low ($p = 0.08$). For the *PPARG* Pro12Ala polymorphism, contrary to our findings, several other authors did not find an association between the polymorphism with the risk of breast and lung cancers.[9,22] For the *IL6*-174G/C promoter polymorphism, our finding was in partial agreement with Campa et al[22] who showed no association between the polymorphism and nonsmall cell lung cancer in general (although the association became apparent when squamous cell carcinomas of the lung were analyzed separately). To our knowledge, this is the first report on the association of the two polymorphisms and ovarian cancer risk.

We also investigated the association between E-selectin S128R (A561C) polymorphism and ovarian cancer risk. We showed that the variant allele of the polymorphism was associated with an increased ovarian cancer risk. E-selectin is a calcium-dependent cell adhesion molecule which is

Table 4 Combinations of *NFKB1* and *NFKBIA* polymorphism and their association with ovarian cancer risk.

NFKB1 genotype	*NFKBIA* genotype	Case ($n = 687$)	Controls ($n = 687$)	OR (95% CI)	*p*
Del/del	CC	161	172	Reference	—
Del/ins	CC	246	241	1.09 (0.83–1.44)	0.54
Ins/ins	CC	79	65	1.30 (0.88–1.92)	0.19
Del/del	CT	59	72	0.88 (0.58–1.31)	0.52
Del/ins	CT	93	93	1.07 (0.75–1.53)	0.72
Ins/ins	CT	29	25	1.24 (0.70–2.21)	0.47
Del/del	**TT**	**1**	**9**	**0.12 (0.01–0.95)**	**0.04**
Del/ins	TT	12	5	2.56 (0.88–7.44)	0.08
Ins/ins	TT	7	5	1.50 (0.47–4.81)	0.50

Significant *p* values are shown in bold.

involved in the extravasation of leukocytes during inflammation. It has been shown that many metastasizing cells exploit the expression of E-selectin to extravasate from the microvasculature.[23] The interaction between E-selectin and its ligands could activate several signaling pathways, such as the MAPK pathway, which confer selective survival advantage for cancer cells.[24] The variant allele of the E-selectin S128R (A561C) polymorphism results in an increased affinity of E-selectin for its ligands.[25] Hence, the variant allele could be associated with an increased cancer risk, which explains our observations in the current study. Similar to our study, Xia et al[12] reported that the variant allele of the polymorphism was associated with an increased risk of gastric cancer in a Chinese population. In addition, several previous studies also demonstrated that the variant allele was associated with poor prognosis of colorectal and breast cancers.[26,27] This is the first report investigating the association of E-selectin polymorphism with the risk of ovarian cancer.

NFKB1 and *NFKBIA* encode for nuclear factor-kappa B (NF-κB), and its inhibitor, IκBα, respectively. NF-κB has been thought to be the master regulator in the inflammatory pathway. Therefore, the polymorphisms in *NFKB1* and *NFKBIA* genes could present differential risk of various cancers in different individuals. We investigated the association of *NFKB1*-94 ins/del polymorphism and *NFKBIA*-826C/T polymorphism with ovarian cancer risk. We found that the ins/ins genotype of the *NFKB1* polymorphism was associated with an increased ovarian cancer risk. This is explained by the fact that the ins allele causes a higher transcriptional activity, which eventually produces a higher level of its protein product that mediates the inflammatory pathway. Our findings agree with those of Fan et al[13] and Suzairi et al[14] but disagree with those of Andersen et al[28] and Riemann et al[29] probably due to the different genetic backgrounds of the study participant included. For the *NFKBIA* polymorphism, we found no association of the polymorphism and ovarian cancer risk. This is consistent with the findings of Tan et al[15] but disagrees with the findings of Lin et al.[30] We also investigated the association between combinations of various *NFKB1* and *NFKBIA* genotypes with ovarian cancer risk. Interestingly, we showed that out of all the possible combinations, only the *NFKB1* del/del + *NFKBIA* TT combination was associated with ovarian cancer risk. This highlights the complex interaction between *NFKB1* and *NFKBIA* in modulating cancer risk.

Intercellular adhesion molecule-1 (ICAM-1) is a cell adhesion molecule which also plays a pivotal role in inflammatory responses. Specifically, it facilitates the transport of leukocytes to inflammatory sites, which further contributes to the instability of the tumor environment. We investigated the association between *ICAM-1* K469E polymorphism and ovarian cancer risk, and showed the presence of an association between the heterozygous KE genotype and a decreased ovarian cancer risk. This is inconsistent with the findings of Tian et al[10] who found that the KK genotype was associated with an increased gastric cancer risk, as well as the findings of Cai et al[16] who showed that the variant EE genotype was associated with an increased the risk of ovarian cancer. In our study, the variant EE genotype was not associated with ovarian cancer risk. This suggests that the risk modification effect of the variant allele was not dose dependent.

Conclusion

We have successfully established the association of *PPARG* Pro12Ala polymorphism, *IL6*-174G/C polymorphism, E-selectin S128R (A561C) polymorphism, *NFKB1*-94 ins/del polymorphism, *NFKBIA*-826C/T polymorphism, and *ICAM-1* K469E polymorphism with ovarian cancer risk in a Chinese population. Our results showed that the *PPARG* Pro/Ala genotype, E-selectin AC genotype, and *NFKB1* ins/ins genotype were associated with an increased ovarian cancer risk in the population studied. In addition, the *ICAM-1* KE genotype was associated with a reduced ovarian cancer risk. However, no association was found between the *IL6* and *NFKBIA* polymorphisms and ovarian cancer risk. Information regarding genetic associations is only valuable when functional assays are performed to investigate the role of the genetic variation in carcinogenesis, or when the associations are replicated in independent cohorts. The former approach is time-consuming, and requires manpower and budget, therefore, the present study focused on the replication of the associations in independent cohorts. Out of the four polymorphisms found to be associated with ovarian cancer risk in our study, only two (i.e., *NFKB1* -94 ins/del and *ICAM-1* K469E) had been previously demonstrated in ovarian cancer.[13,16] These two polymorphisms could serve as genetic biomarkers for ovarian cancer, while the utility of the other four polymorphisms as genetic biomarkers are waiting for further confirmation in independent cohorts of ovarian cancer.

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